ademtech

Bio-Adembeads Protein G 0433
for qualitative and fast results

For research use only

INTRODUCTION

Protein G is a cell wall component produced by group G *Streptococci*. It's a Type III Fc receptor that binds to the Fc region of IgG by non-immune mechanism similar to that of protein G from *Staphylococcus aureus*.
The protein G employed is a recombinant form expressed in *Escherichia coli*. This form laks albumin and Fab binding sites and membrane-binding regions.

PRODUCT DESCRIPTION

Bio-Adembeads Protein G are uniform sized superparamagnetic nanoparticles conjugated with protein G.
They are produced and supplied in an aqueous suspension containing 0.05% Proclin 300.

PHYSICAL CHARACTERISTICS

Diameter: 300 nm (CV max 20%)

Magnetic susceptibility: approx. 40 emu/g

Specific surface area: 10 m^2/g

Iron oxide content: approx. 70%

Binding capacity : ~100µg/ml

BIO-ADEMBEADS PROTEIN G PRINCIPLE

Immunoprecipitation:

Bio-Adembeads Protein G can be used for immunoprecipitation. In this case, cells are lysed and cell debris are removed.A specific antibody is first added to the cell lysate. The Bio-Adembeads protein G are then incubated with the cell lysate in order to form a magnetic labelled immune complex wich can be easily separated from the liquid by removing the supernatant during the magnetization step.

Immunoglobulin purification:

Bio-Adembeads Protein G are particularly suitable for immunoglobulin purification from ascite, serum, tissue culture supernatants or other samples. After a short incubation time the immunoglobulins are eluted from the Bio-Adembeads Protein G and the containing Ig supernatant is pipetted off for downstream applications.

Species	Affinity for Protein A	Affinity for Protein G
Chicken	-	+
Cow	++	++++
Goat	-	++
Guinea Pig	++++	++
Hamster	+	++
Horse	++	++++
Human IgG_1	++++	++++
Human IgG_2	++++	++++
Human IgG_3	-	++++
Human IgG_4	++++	++++
Mouse	++	++
Mouse IgG_1	+	++++
Mouse IgG_{2a}	++++	++++
Mouse IgG_{2b}	+++	+++
Mouse IgG_3	++	+++
Pig	+++	+++
Rabbit	++++	+++
Rat	+/-	++
Rat IgG_1	-	+
Rat IgG_{2a}	-	++++
Rat IgG_{2b}	-	++
Rat IgG_{2c}	+	++
Sheep	+/-	++

Table 1: Protein A/G affinities for IgG from various species

INSTRUCTIONS FOR USE

IMMUNOPRECIPITATION

A. Before starting

The choice of lysis buffer is critical and dependent on the nature of the protein to be studied. The RIPA buffer or NP 40 buffer could be used in most cases.

RIPA Buffer gives the lowest background, but can denature some kinases. It also has the potential to disrupt protein:protein interactions. In this case, we strongly recommend to homogenize the cell lysate by passing through a needle (23G, 0.6mm) prior adding antibody or to sonicate and centrifuge 10' at 10000 rpm and keep the supernatant.

NP40 (newly Igepal CA 630) Buffer is less denaturing, but gives a higher background. In order to reduce kinase activity inhibition and protein complexe disruption, prefer using this buffer.

Buffer composition

RIPA Buffer	50mM Tris, 150 mM NaCl, 1% Triton X100, 1 % Deoxycholate, 0.1% SDS, pH 8
NP40 Buffer	20mM Tris, 150mM NaCl, 0.5 % Deoxycholate, 0.5 % NP40, pH8

Notes:

- The lysis conditions must be carefully chosen. Increase the salt concentration, decrease the detergent concentration, or change the detergent can optimized conditions for immunoprecipitation.
- To prevent protein degradation, we recommend performing lysis on ice and or adding proteinase inhibitors to the lysis buffer.

B. Immunoprecipitation Procedure

The protocol below offers a general guideline for immunoprecipitation. Optimization may be required for each antigen and antibody. The optimal amount of antibody depends on individual antibody used.

For optimal performance, match the amount of Bio–Adembeads Protein G to the amount of the antibody to be captured.

Bio-Adembeads Protein G	Antibody
20µl	2µg
40µl	4µg
60µl	6µg

Table 2: Ratio Beads/Antibody

The following protocol is established for 20µl Bio-Adembeads Protein G and 2µg of monoclonal antibody. If you use more beads, proportionally adjust all the volume of buffers.

B.1. Basic Protocol (without IgG Cross-linking)

1. **Add 2 µg of monoclonal antibody** to 500µl of cell lysate (corresponding to 1-10 $x10^6$ cells)**.**
2. **Incubate 30 min minimum** under agitation (~1000 rpm) at room temperature**.**
3. **Bio-Adembeads Protein G washing procedure.**

3.1. Pipette and transfer **20µl of Bio-Adembeads Protein G** into a test tube.

3.2. Place the tube on a magnet until pellet forming. Pipette off the supernatant carefully, leaving beads undisturbed.

3.3. Remove the test tube from the magnet and resuspend the beads carefully in **20µl of Lysis buffer**.

3.4. Repeat one time steps 3.2 and 3.3.

4. **Binding of Bio-Adembeads Protein G to immune complex**

4.1. Add **20µl of washed Bio-Adembeads Protein G** to the immune complex.

4.2. **Incubate 30 min minimum** under agitation (~1000 rpm) at room temperature.

Notes:

- Depending of the affinity of the antibody, increasing the incubation time could increase yield.
- For sensitive proteins, incubation must be run at 4°C.
- Note that some proteins have the ability to interact with Protein A/G without any antibody as a bridge[1]. As negative control, use Bio-Adembeads Protein G complexed with non relevant antibody.

5. **[Target-Protein–beads] complex washing procedure**

5.1. Place the tube on a magnet until pellet forming, discard the supernatant, remove the test tube from the magnet and add **500µl of Lysis Buffer**. Be sure to resuspend the beads by pipetting several times.

5.2. Repeat 3 times step 5.1.

5.3. Place the tube on the magnet until pellet forming, discard the supernatant, remove the test tube from the magnet and resuspend the beads in **20µl of Lysis Buffer**.

To avoid non specific binding, be sure to homogenize the beads by pipetting.

6. Bio-Adembeads Protein G target protein elution procedure

To elute conventional methods could be applied:

-Lower the pH to about 2.0–3.5,
-or increase the pH to about 11.

The method of choice depends of the target protein, the downstream applications and detection methods.

As a general method, we recommend 50mM glycine, 0.65 % Tween 20, pH 2.7 as elution buffer or to use PAG Elution Buffer (#10701).

6.1 Place the tube on the magnet until pellet forming. Pipette off the supernatant.

6.2. Remove the test tube from the magnet and **add 15µl of Elution Buffer**. Mix well by pipetting during 2min.

6.3. Place the tube and the magnet until pellet forming and **keep the supernatant** containing the target protein.

As a safety measure to preserve the activity of acid labile IgGs, we recommend adding basic buffer to neutralize neutralize the eluted fraction (e.g. Tris, pH 7.5).

Notes:

- If necessary a second elution could be performed (Repeat step 6.1 and 6.2).
- Elution volume of 15µl can be used until 100µl of beads.
- Beads magnetization (step 6.3) is important to avoid contamination.
- Reduction of disulfide bridges occurs with alternative elution methods (e.g SDS, DTT). Avoid using these methods after cross-linking procedure, use Elution PAG Buffer (#10701) to prevent antibody release.
- With low affinity antibody, using elution buffer containing SDS could increase yield, but also increase the release of non specific protein complexes with Protein A/G[1]. PAG elution buffer (#10701) is sufficient for most application.

B.2. IgG Cross-linking Protocol

If coelution of immunoglobulin is not desired, a chemical crosslinking between specific antibodies and the Protein G coated on the surface of the Bio-Adembeads can be performed.

1. **Pipette and transfer 20µl of Bio-Adembeads Protein G** into a test tube**.**
2. Place the tube on a magnet until pellet forming. Pipette off the supernatant carefully, leaving beads undisturbed**.**
3. Remove the test tube from the magnet and **resuspend** the beads carefully in **20µl of PBS 0.65% Tween 20 pH 7.5.**
4. Repeat one time steps 2 and 3.
5. **Add 2µg of monoclonal antibody** and **incubate 10-30 min** minimum under agitation (~1000 rpm) at room temperature**.**
6. Place the tube on the magnet until pellet forming and discard the supernatant. Remove the test tube from the magnet and resuspend the beads **in 20µl of PBS 0.65% Tween 20 pH 7.5.**
7. Repeat one time steps 6**.**
8. Place the tube on the magnet until pellet forming. Pipette off the supernatant and resuspend the beads in **100µl of 20mM DMP** (dimethyl pimelimidate dihydrochloride) **dissolved in 200mM Triethanolamine pH 9.**

This DMP solution has not a long lasting stability. It must be prepared just before adding to the beads.

9. **Incubate 30 min minimum** under agitation (~1000 rpm) at room temperature**.**
10. Place the tube on a magnet until pellet forming and discard the supernatant**.**
11. Remove the tube from the magnet and stop the reaction by adding **20µl of 50mM Tris pH 7.5** and **incubate 15min minimum** under agitation (~1000 rpm) at room temperature**.**
12. Place the tube on a magnet until pellet forming and discard the supernatant**.**
13. Remove the tube from the magnet **and resuspend** the beads **in 20µl of 50mM glycine, 0.65 % Tween 20, pH 2.7.**
14. Repeat one time steps 13 and 14**.**
15. Place the tube on the magnet until pellet forming, discard the supernatant, remove the test tube from the magnet and resuspend the beads in **20µl of Lysis buffer.**
16. Add the beads to the cell lysate to perform your immunoprecipitation**.**
17. Proceed to section B1 step 4.2 and follow the procedure**.**

IMMUNOGLOBULIN PURIFICATION

1. Pipette **20µl of Bio-Adembeads Protein G** into a test tube**.**
2. Place the tube in a magnet until the pellet forming Pipette off the supernatant carefully, leaving beads undisturbed.
3. Remove the test tube from the magnet and **resuspend** the beads carefully **in 20µl of PBS 0.65% Tween 20 pH 7.5.**
4. **Repeat one time step 2 and 3.**
5. **Add 20 µl of washed Bio-Adembeads Protein G per 10µl of diluted serum** (dilution 1/10 in PBS 0.65% Tween 20 pH 7.5).
6. **Incubate 10-30 min** minimum under agitation (~1000 rpm) at room temperature**.**
7. Place the tube on a magnet until pellet forming and pipette off the supernatant**.**
8. Remove the test tube from the magnet and resuspend the beads in **20µl of PBS 0.65% Tween-20 buffer pH 7.5.**
9. Repeat twice steps 7 and 8**.**

For viscous samples this step can be repeated and the volume of washing could be increase.

10. To elute, proceed to section B1 step 6**.**

ADDITIONAL MATERIAL REQUIRED

- PAG Elution Buffer (#10701)
- Magnetic devices
  - Adem-Mag SV, 1.5 ml (# 20101)
  - Adem-Mag MV, 15 ml (# 20102)
  - Adem-Mag HV, 50 ml (# 20103)
  - Adem-Mag MODULO (# 20105)
- Rotation device
- Test tubes

STORAGE / STABILITY

When stored in unopened vials at 2-8°C, Bio-Adembeads Protein G are stable until expiration date printed on the label.
The Bio-Adembeads Protein G must be maintained in liquid during storage and all handling steps. Drying will result in reduced performance. Do not freeze the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination of protein-coated Adembeads.
If cytotoxic preservatives are added they must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For in vitro research only. Not for use in human diagnostic or therapeutic procedures.

Avoid pipetting by mouth.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.
Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

Ademtech SA – Bioparc BioGalien - 27, allée Charles Darwin - 33600 PESSAC – FRANCE
www.ademtech.com

[1]Immunoprecipitation alert, L Luand, Y Wang, Cell Cycle, 2008, 7(3), 417-8